THE ROLE OF MACROPHAGES IN IMMUNOLOGY

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Macrophages play a significant part in immunity and immune responses. They assume a defensive role exhibited by their ability to carry on phagocytosis of parasites and microbes. They regulate lymphocyte activation and proliferation and they are essential in the activation process of T- and B-lymphocytes by antigens and allogenic cells. Enhanced bactericidal activity of "activated macrophages" is based on immunologically linked mechanisms involving lymphocytes. Macrophages kill ingested microbes but the mechanism by which this is accomplished is not completely understood. This paper discusses the role of macrophages in relation to immunity.

BIOLOGY OF MACROPHAGES

Macrophages are mononuclear cells capable of phagocytosis. They are distributed throughout mammalian organs and their morphology varies depending on their state of activity. Peritoneal macrophages (in Giemsa-stained or May-Grünwald-Giesma stained preparations) measure 10 to 30 μm in diameter. The cytoplasm contains vacuoles and is slightly basophilic. The nucleus is ovoid and measures 6 to 12 μm in diameter. By phase contrast microscopy, peritoneal macrophages contain light gray diffuse cytoplasm with dark gray rod-shaped mitochondria. Granules and vacuoles are seen depending on the physiological state of the cell.[1]

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North and Mackaness[2] found that normal peritoneal macrophages from nonimmunized mice had a cytoplasm enclosed by a three-layered membrane 80 A thick with many protuberances and invaginations due to a high degree of activity. The periphery of the cytoplasm was finely granular and lacked structures such as endoplasmic reticula, with and without attached ribosomes, and cylindrical mitochondria. These structures were found in the rest of the cytoplasm rather than at the periphery. Three types of cytoplasmic vesicles were seen ranging from 300 A to 0.5 μm in diameter and enclosed by a unit membrane. The three types of vesicles were small pinocytic vesicles, various sized organelles containing a fine granular material and a larger, denser vacuole. Ribosomes were attached to the external portion of the nuclear membrane which was continuous with the endoplasmic reticula.

Various types of stimuli may cause "macrophage activation." Examples are stimuli which accompany phagocytosis of bacteria. Activated macrophages are metabolically highly active and contain organelles such as lysosomes rich in hydrolytic enzymes. North and Mackaness[2] observed that following ingestion of Listeria monocytogenes by mouse peritoneal macrophages, the bacteria within phagocytic vacuoles were surrounded at first by a clear area bounded by a unit membrane and later the clear area was filled with an amorphous material which apparently had been transferred from cytoplasmic vesicles (lysosomes) into the phagosomes to form phagolysosomes. In other studies it was observed that there is a limit to the volume of particles taken up, and that this either imposes or is associated with a limit to the increased rate of respiration. As glycolysis is considered to be responsible for the energy needed for particle uptake in the macrophage, it is unlikely that a limit to the rate of oxygen consumption sets the limit to the number of particles taken up.[3]

Peritoneal macrophages of mice that survived an initial infection with L monocytogenes were regarded as "immune" because they could kill the specific organism rather than support its intracellular growth. Macrophages from mice infected with Mycobacterium bovis, strain BCG, were observed to be refractory to infection by unrelated pathogens in vitro.[4] In addition, such macrophages were found to have greater in vitro chemotactic response than the response of those from uninfected mice.[5] North and Mackaness[6] compared the ultrastructure of peritoneal macrophages from mice immunized with L monocytogenes with that of peritoneal macrophages from nonimmunized mice. They observed that the cytoplasmic membrane of immune macrophages was smoother, and had fewer protuberances and invaginations than the membrane of nonimmunized macrophages. Immune macrophages contained many free ribosomes but very few profiles of endoplasmic reticula. The mitochondria were smaller, more numerous and contained more cristae. The cytoplasm of immune macrophages was less dense and appeared to be highly dehydrated. It contained fewer vesicles and many Golgi bodies. Dumont and Sheldon[7] found similar results using peritoneal macrophages from nonimmunized hamsters. The morphology of mouse and hamster peritoneal macrophages was significantly different from that of other mammalian and avian peritoneal macrophages. Alveolar macrophages were morphologically distinct from peritoneal macrophages. Leake and Heise[8] observed these differences with light and electron microscopy. They found that the nuclei of alveolar macrophages were round or slightly ovoid and nucleoli were seen more often than in peritoneal macrophages. Peritoneal macrophages had elongated, deeply indented nuclei. Rough endoplasmic reticulum was frequently seen in peritoneal macrophages, but rarely in alveolar macrophages. Peritoneal macrophages had more Golgi bodies than did alveolar macrophages. Alveolar macrophages had dense cytoplasmic granules not observed in peritoneal macrophages. Mitochondria from peritoneal macrophages were larger and more elongated than mitochondria from alveolar macrophages and the overall diameter of alveolar macrophage was approximately 1.5 times that of the peritoneal macrophage.

Kajita et al[9] studied the alveolar macrophages of soot-exposed mice. These workers found that the mice that inhaled soot particles had clear membrane-bound vacuoles. The cells contained eccentric rounded nuclei in an abundant low-density cytoplasm. The mitochondria were elongated with regularly arranged cristae and the cell membrane showed invaginations. Karrer[10,11] reported similar observations on the morphology of normal mouse alveolar macrophages and those that had phagocytized India ink. Splenic macrophage morphology was similar to peritoneal macrophages.

MACROPHAGE AND IMMUNITY

Studies have shown that mouse macrophages obtained after intraperitoneal stimulation with thioglycolate medium accumulated and secreted high levels of plasminogen activator in culture whereas macrophages obtained from unstimulated mice did not.[12,13] Hibbs[14] reported that activated macrophages from mice with chronic toxoplasma infection are capable of destroying tumorigenic target cells in vitro.

Blood monocytes can mature into tissue and exudative macrophages. Volkman and Gowans[15] conducted radioautographic studies and found evidence which suggested that blood monocytes accounted for many macrophages in local inflammatory sites.

Gough et al[16] demonstrated that pure preparations of blood lymphocytes did not transform into macrophages during culture in vitro, but when neutrophils were added in small aliquots, lymphocyte macrophage transformation occurred. This suggested that the lymphocyte to macrophage transformation occurred in vivo depending on the homeostatic control by neutrophils or their degradation products.

Jones[17] produced evidence that neutrophils stimulate macrophage differentiation. This was demonstrated by mixing cultures with genetically similar blood leukocytes. Many macrophages were present at three days and numerous blast cells were seen at six to nine days. When neutrophils were removed before the mixed cultures were prepared, small numbers of macrophages were found. Jones[17] reported that the structure of macrophages varies with the length of time in culture. The phases in its culture were described as adherence, spreading and phagocytosis of debris, mitosis, and extended culture. The shape of peritoneal macrophages was observed during the process of settling on a glass surface using scanning electron microscopy. During prolonged culture the shape was flattened and approximately circular, elongated, or fully extended measuring 15 to 18 μm.[18]

Carr[1] stated that macrophages had to be able to move in order to carry out their biological functions, and that the process of extension onto glass was regarded as a form of movement. Once this was over, they pushed pseudopodia out in various directions and pulled them back again. It was reported that actual change in position was scanty or absent and the rate of movement of macrophages in culture depended on the origin and cultural technique but did not give an indication of macrophage movement in the body.[1] Chemotaxis is a reaction whereby the direction of locomotion (of cells) is determined by chemical substances in the environment. It is often described as being positive or negative according to whether the movement is towards or away from the substance in question. Keller and Storkin[19] showed that the chemotactic effect of many substances, including antigen-antibody complexes, endotoxins, and bacteria, were due to the formation of mediators which act directly on cells (cytotaxins). Many substances react with normal serum to release cytotaxins. Distinct specific cytotaxins exist for polymorphs and mononuclear cells. It was demonstrated that factors in normal rabbit serum caused directional migration of both neutrophils and mononuclear cells.[1] The movement of neutrophils was increased by the addition of antigen-antibody complexes to the serum, while that of monocytes was reduced.[20] George and Vaughan[21] showed that migration of macrophages was inhibited both in tuberculin hypersensitivity and hypersensitivity induced by immunization with a protein in Freund's adjuvant.[22-24] Inhibition of migration was specific and due to an effect exerted by a relatively small number of sensitized cells. Bennett[18] stated that it was likely that the sensitized cells were lymphocytes and that they released a substance, probably a protein, into the medium; this inhibited the migration of macrophages. There was evidence that a migration inhibition factor might be produced by polymorphs.[25]

Boyden and Sorkin[26] were the first to coin the term "cytophilic" to designate affinity for cells. These authors described cytophilic antibodies formed from rabbits in response to human serum albumin. In the free state these antibodies had an affinity for certain cells in a mixed population of cells from the spleen of normal rabbits. It was also reported that the sites on antibodies responsible for their affinity for cells were independent of the sites which bound antigen.[26]

Boyden[27] reported that phagocytes that dispose of foreign material must have a recognition mechanism for distinguishing between these materials and the viable cells of itself. In addition, humoral recognition factors (antibodies) fulfilled this role, either by coating the particle to be phagocytized or by first attaching to the phagocyte. It was shown that humoral antibodies of certain classes could be absorbed by membranes of macrophages before combining specifically with antigen. Thus, the macrophages probably contained surface specific recognition factors in the form of cytophilic antibodies which promoted the adherence and phagocytosis of antigen bearing particles. Benacerraf[28] postulated that under proper conditions all cytophilic antibodies might act as opsonins to promote phagocytosis. Boyden[29] produced antibodies cytophilic for macrophages by administering sheep red blood cells (SRBCs) in Freund's complete adjuvant to guinea pigs. The resulting antibodies combined with receptors on macrophages and were then able to bind SRBCs to give the appearance of a rosette, this binding was considered a prerequisite to efficient phagocytosis.

Carr[1] reported that macrophage cytophilic antibodies vary in characteristics depending on factors such as species of origin and the route and schedule of antigen administration and that within a species, macrophage cytophilic receptors for IgG and IgM antibodies might differ.

Nelson and Mildenhall[30] used SRBCs in Freund's complete adjuvant to produce both delayed hypersensitivity and cytophilic antibody in guinea pigs. When the antigen emulsion was administered intraperitoneally, intradermally, or into the foot pads, high titers of macrophage cytophilic antibodies were obtained in two weeks; the intradermally injected animals also exhibited pronounced delayed reactions.

Carr[1] reported that macrophages engulfed a volume greater than their starting volume and as this occurred, the cytoplasm of the macrophage became more voluminous, thus increasing their size and capacity. The process of phagocytosis was described as an energy-consuming process and part of consumed energy was utilized in the manufacturing of phospholipid which was necessary for making new cell surface membranes and phagocytic vacuoles. Carr[1] also reported that phagocytosis might involve ingestion, digestion, sequestration, and ejection. These processes were outlined as follows: phagocytosis first involved contact of the cell's plasma membrane with the material to be ingested. The material attached to the macrophage surface and was brought in. This material first lay within an indented plasma membrane and the membrane continued to invaginate further, forming a deep recess at the cell surface. The material became continuous with the plasma membrane by a narrow neck and then detached from the inside surface of the cell membrane, becoming free in the cytoplasm as a phagocytic vacuole or phagosome which moved centrally. Lysosomes containing hydrolytic enzymes moved to the phagosome and the lysosomal and phagocytic membranes fused and disintegrated causing the phagocytic vacuole and lysosome to coalesce and form a heterolysosome. As a result, the lysosomal enzymes were mixed with the engulfed material and were set to digest it.[1] Digested material was eventually reduced to dense bodies of varied composition. These bodies fused into large irregular masses and placed in a peripheral part of the cell to be pinched off and thus separated from the cell. Macrophages further digested their phagocytic load and tended to isolate it in the cytoplasm. This was termed sequestration apparatus. The cell might then eject this material.

In addition to the presence of hydrolytic enzymes in lysosomes, Weiss[20] stated that macrophages had other enzymes also found in lysosomes and among these were acid phosphotase, cathepsin, β-glucoronidase, acid ribonuclease, acid deoxyribonuclease, aryl sulphatase, lipase, and as mentioned previously, lysozymes. Some hydrolytic enzymes were found to appear through the cytoplasm, were not membrane bound, and included nonspecific esterase and lipase.[20]

Weiss[20] reported that enzymes were not necessarily confined within the vital macrophage because the fluid surrounding the macrophages was enzyme rich and that the macrophage itself secreted enzymes.

Additional enzymes associated with macrophages included cholesterolesterase utilized for breaking down cholesterol and that the macrophages manufacture phospholipids in relationship to cholesterol breakdown and the synthesis of new membranes.[20]

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